Manual

Installation

To install the application unzip the downloaded files to a location of your preference. Afterwards, add the installation directory to your Matlab path, e.g. by

```
addpath('path/to/installation')
```

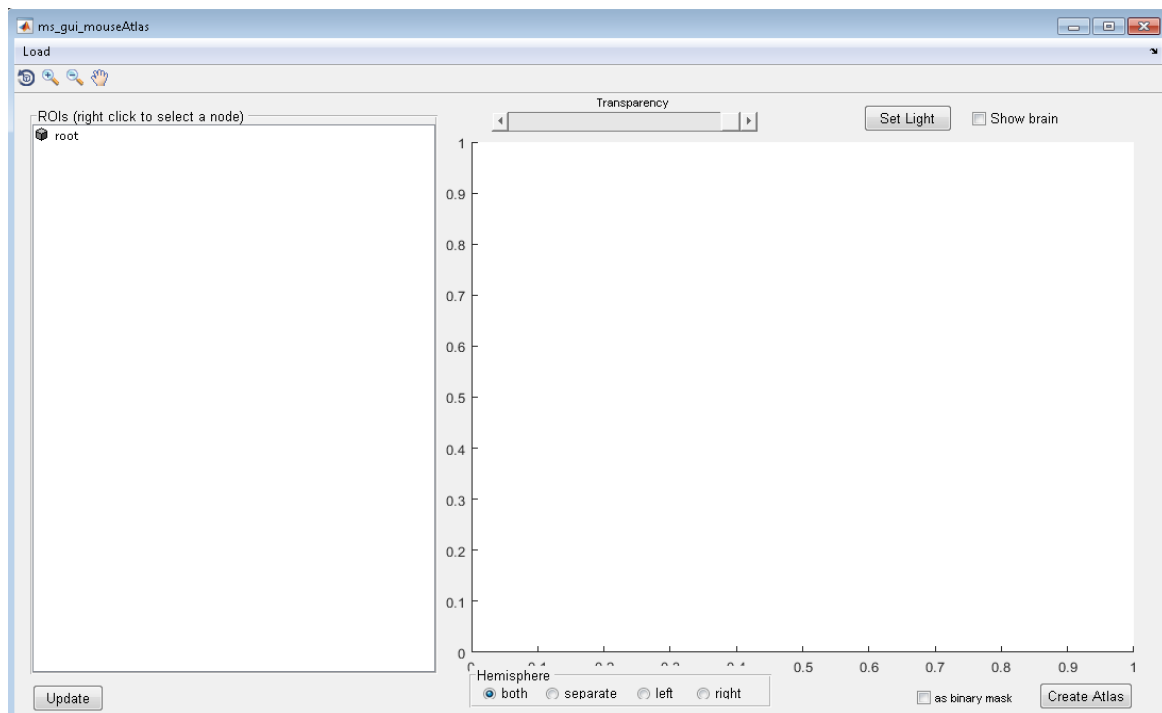You also need to have SPM12 installed and added to your Matlab path.

Usage

Start

After setting the paths you can start the application by typing

```
ms_gui_mouseAtlas
```

in the Matlab command window. The graphical user interface (GUI) should start and you are presented with the following interface:

The left ROI panel (region of interest) is an interactive tree consisting of the available ROIs. A double-click on 'root' (or any node) opens it. To select a ROI for later use, **right-click** on it and the selected ROI is in green, bold font indicating a ROI selection.

On the right: an axis element for 3D illustration of selected ROIs colored corresponding to the ROI icons of the tree selection. Click the 'Update' button to update the 3D illustration. Use the icons under the menu bar to rotate, zoom etc. Above the axis there are some controls to modify the visualization:

- For better visibility ROIs can be made transparent by the 'Transparency' slider. First, left-click on a ROI to select it (it will get highlighted; e.g. a blue background on windows systems or gray in linux) and then use the slider. This helps to see hidden ROIs.
- The 'Set Light' button rearranges the virtual light source.
- The 'Show brain' checkbox illustrates the outline of the brain (outer boundaries of the atlas) in addition to the selected ROIs. Its only purpose is to help visualizing the location of selected ROI(s) and it is not taken into account in the atlas creation.

Creating an Atlas

First, select the ROI which you would like to include in the atlas. You select ROIs by **left-clicking** the corresponding field in the tree selection (you can double click on a branch to open it). After selecting a ROI its text becomes green with a bold font. To unselect a ROI left-click it again.

After the selection is made, you can get a visual representation via the 'Update' button. The color of the shown ROIs corresponds to the coloring of the ROI field in the tree selection (see figure below). Please keep in mind, that this representation is not exact (e.g. very small ROIs are sometimes not visualized).

The figure below is an example of how it may look like.
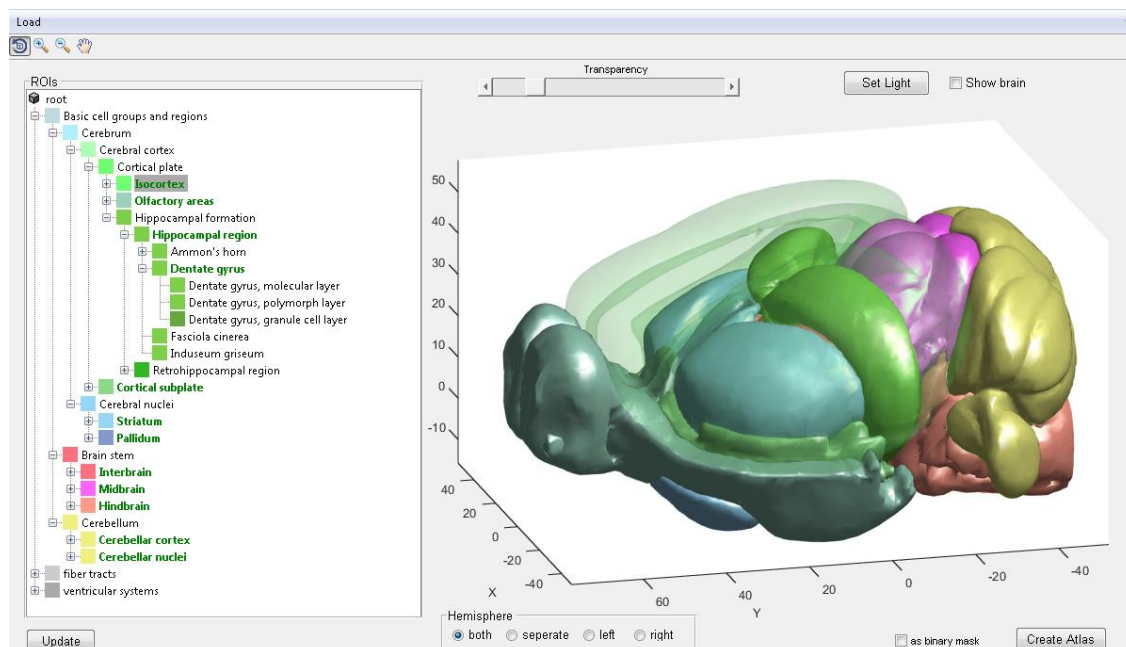
To create the atlas click the 'Create Atlas' button and choose a location to save it. The app generates three files:

1. The annotation text file in which the name of a ROI is linked to its numeric value in the atlas nii-file.

2. The atlas itself in Allen atlas space.
3. The atlas (file as in 2.) transformed into Paxinos space (suffix '_inPax').

That's it. ☺

When the file operation is done, an SPM graphics window appears presenting the created atlas and the average template which was provided by the Allen Institute. Here, you can check again the atlas and the ROI definition (see the figure below).

newAtlas.nii
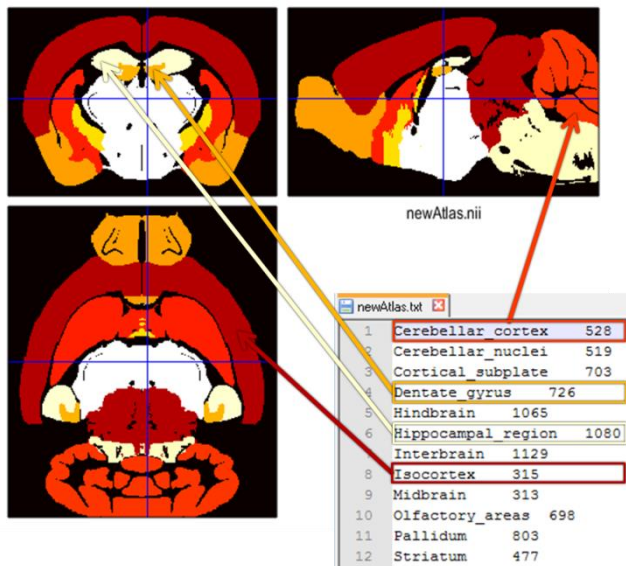
When checking the atlas, make sure that 'Interpolation' is set to 'NN'. Changing the colormap to something different from the standard 'Gray' is helpful (menu 'SPM Figure' -> 'Colours' -> 'Colormap' -> …) e.g. 'Hot' as in the example figure at the left.

	newAtlas.txt	
1	Cerebellar_cortex	528
2	Cerebellar_nuclei	519
3	Cortical_subplate	703
4	Dentate_gyrus	726
5	Hindbrain	1065
6	Hippocampal_region	1080
7	Interbrain	1129
8	Isocortex	315
9	Midbrain	313
10	Olfactory_areas	698
11	Pallidum	803
12	Striatum	477

Useful Functions

Load an already defined atlas

The app has a menu. Click on 'Load' and choose the annotation .txt file of a previously created atlas to auto-select the corresponding ROIs. From there you can change your selection and create a new atlas.

Save as binary mask

You can save ROIs as binary masks (consisting of 1s and 0s). To do so, check the 'as binary mask' option and create the atlas. The .nii files are binary masks of the selected ROI. The .txt file states which ROI was used to create the mask. In case you selected several ROIs they get combined to one resulting ROI.

Choose hemispheres

The app allows selecting different hemispheres (see the 'Hemisphere' panel). The default option is 'both', meaning that ROIs cover both hemispheres. In case you need only the left or right hemisphere, choose the corresponding button. In case you need ROIs separated by hemispheres select 'separated' (Note: To achieve this functionality, the original atlas is simply split in half. Check the atlas afterwards! Furthermore, a necessary renumbering of the ROIs takes place, so that you won't be able to load the atlas in future sessions. Best practice would be to save the atlas again using the 'both' option.).

Selecting 'Sub-ROIs'

The app allows selecting ROIs on any level of the hierarchical structure of brain regions like in the example above in which the 'Hippocampal region' and the 'Dentate gyrus' are selected as ROIs. The latter is a sub-ROI of the former, and thus, there is a parent-child relationship. This 'feature' is uncommon in other atlases and enables a quick and easy finer ROI analysis, e.g. a single analysis of a sub-ROI or even excluding a certain sub-ROI of a 'parent' without the need to redefine the atlas. But it also contains a certain danger, since the corresponding parent-ROI definition is missing this sub-region. Therefore, the user should be aware and keep in mind his/her selection of parent and child ROIs! As in the example above, the 'Hippocampal region' does not include the 'Dentate gyrus' ROI anymore, so that its child 'Dentate gyrus' has to be included in case the complete parent ROI 'Hippocampal region' is of interest.

This danger could be overcome by a renaming of parent-ROIs. But the developers decided against it because of its inherent complexity (imagine the definition of 'sub-sub-ROIs' and its implications), the inconsistency to previously created atlases and the possibility to break workflows in case they depend on ROI names.

For Advanced Matlab Users

Feel free to modify the code as specified by your needs. The authors tried to make the app as comprehensive as possible but could not test every environment.

GUI modifications

Depending on your screen resolution or operating system you may find panels, button groups or the GUI itself being too big or too small. The GUI was created by MATLAB's 'guide' function. Thus you can change the GUI by the following command:

```
guide ms_gui_mouseAtlas.fig
```

Changing the applied transformation

The transformation of the atlas into the Paxinos template space is a SPM12 job batch which applies a deformation field to the initially created atlas (which is originated in the Allen space). To create this field we used the 'old normalize' function in SPM12 with an anatomical template in Paxinos space as 'template' and a simulated anatomical image in Allen space as 'source'. The simulated image is in principle just an atlas in which GM, WM, and CSF ROIs are defined. To mimic an actual anatomical image, you can apply values to the ROIs which are similar to the corresponding intensities of your anatomical template, e.g. by using spm_imcalc (this seems to improve the normalization routine).

You might like to change this transformation to fit the workflow of your lab. A mouse brain template can be lab- and/or study-specific and the provided transformation might not fit your needs.

After you obtained your own deformation field you can change the line in the transformation batch pointing to the field (.mat file) which is applied. Open the 'ms_allen2pax_batch.m' file (located in the 'TransJob' folder) and modify the following line:

```
…
%% OldNorm
deformationFile = 'shift2pax_sn_20171221.mat';
```

…

Replace the 'deformationFile' variable with your .mat file.

Disclaimer